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Microdetermination of Carbon and Hydrogen

A Statistical Study of Factors

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A collaborative study of the microdetermination of carbon and hydrogen was conducted to find an accurate method suitable for adoption by the Association of Official Agricultural Chemists. The results were analyzed statistically to determine the effects of different variables on the accuracy and precision of the method. Of the twelve factors considered, those which affected the accuracy of the carbon values most were the size of the sample and the treatment the absorption tubes received before being weighed. None of the factors, however, had much influence on the precision of the carbon values and on the accuracy and precision of the hydrogen values. This statistical analysis indicated that certain details of the carbon and hydrogen method should be eliminated, that others were not necessary, and that a more simplified procedure than is often used should produce more accurate and equally precise results.

THE micromethods for carbon and hydrogen in general use today and their semimicro modifications are all based on the catalytic combustion of the organic material to form carbon dioxide and water, followed by the absorption and weighing of these two products. Although the principle of the methods is the same, the details of the procedures vary from laboratory to laboratory.

A collaborative study was undertaken to determine which of these variations should be included in a standard method for the Association of Official Agricultural Chemists. Twenty laboratories participated in this study and performed 115 carbon and hydrogen analyses on nicotinic acid (sample 1) and 95 on benzylisothiourea hydrochloride (sample 2). Each analyst was asked to perform the analyses by his own method under his normal working conditions and to report all values obtained. Details of the procedures used were obtained from each laboratory by a questionnaire designed to give uniform and fairly complete information.

Analysis of the questionnaires showed that for a number of steps in the procedure two different techniques were used. This made possible a simple division of the data for statistical treatment. Thus, all the data could be classified as micro or semimicro, depending on the sample size, or be grouped according to whether or not some treatment or particular modification of the apparatus was used—for example, were the absorption tubes wiped; was the choking plug omitted?

Table I. Data from Collaborative Study of Carbon and Hydrogen Analysis

Collab. No.	Micro Samples (2–10 Mg.)	Absorption Tubes Wiped	O_2 Replaced with Air	Choking Plug	O_2 Aspirated with Mariotte Bottle	Silver Alone	CuO-Pt Catalysts	Electric Sample Burner	Mechanically Operated Burner	Quartz or Vycor Tubes	Lab Air-Conditioned	Balance Adjacent to Furnace
0	—	—	—	—	—	×	—	×	×	×	×	×
2	—	×	—	—	—	—	×	—	—	—	×	—
10	×	×	?	×	×	×	—	—	—	×	—	×
12	×	×	×	×	×	—	—	—	—	×	×	×
13	×	×	×	—	×	×	×	—	—	—	—	—
17	×	×	×	—	—	—	—	×	×	—	—	—
23	×	×	—	×	—	×	—	—	—	×	—	—
24	×	—	—	—	×	×	×	×	×	×	—	×
27	×	—	×	×	×	×	×	—	—	—	—	×
28	×	×	—	×	—	×	—	—	—	—	—	×
31	×	×	—	×	×	×	×	—	—	×	×	×
35	×	—	—	—	—	×	×	—	—	×	×	×
39	×	—	—	×	—	×	×	×	×	×	×	×
40	×	—	×	—	—	×	×	—	—	×	—	—
41	×	×	×	×	—	×	—	×	—	×	×	—
44	×	—	×	×	×	×	×	×	×	×	—	—
45	×	×	×	×	—	×	×	—	—	×	—	—
46	—	—	—	—	×	×	×	×	×	×	×	—
49	×	×	×	×	×	—	×	×	×	—	×	—
50	×	×	×	×	×	×	×	×	×	×	—	—

× Analyst employed procedure listed at head of column.
— Analyst employed alternate procedure.

Table II. Data from Collaborative Study of Carbon and Hydrogen Analysis

Collab. No.	Carbon Data						Hydrogen Data					
	Sample 1			Sample 2			Sample 1			Sample 2		
	n	$\bar{X}$	log s^2	n	$\bar{X}$	log s^2	n	$\bar{X}$	log s^2	n	$\bar{X}$	log s^2
0	8	58.47	2.6201	8	47.38	1.2788	8	4.10	2.3636	8	5.44	2.4150
2	4	58.44	2.4771	3	47.32	1.8751	4	4.21	1.9823	3	5.57	2.0607
10	8	58.75	2.6739	8	47.43	2.7803	8	4.68	2.1818	8	5.90	2.2900
12	2	58.75	1.9294	2	47.72	0.6990	2	4.10	0.0000	2	5.48	1.1139
13	3	58.99	1.4914	3	47.68	0.8451	3	3.98	1.4314	3	5.35	2.4786
17	8	58.64	2.3201	2	47.44	1.5051	8	4.27	1.9031	2	5.68	2.7619
23	3	58.53	2.0682	4	47.42	2.5079	3	3.97	2.0828	4	5.41	2.5092
24	3	58.64	1.7160	3	47.51	2.1790	3	4.16	1.7853	3	5.56	1.3010
27	3	58.57	1.7076	3	47.48	1.4914	3	4.09	1.4314	3	5.44	1.2304
28	3	58.61	2.7202	3	47.39	2.6702	3	4.04	2.1644	3	5.46	2.4065
31	2	58.40	0.6021	3	47.55	2.5966	2	4.06	1.9912	3	5.37	2.3856
35	8	58.52	3.8790	5	47.51	3.8899	8	4.22	2.3118	5	5.51	2.2672
39	4	58.70	2.7084	4	47.42	2.5276	4	3.95	1.9085	4	5.35	1.9685
40	4	58.62	1.5563	4	47.58	1.4472	4	4.09	1.8633	4	5.48	2.0569
41	5	58.92	1.7853	4	47.66	2.0253	5	4.37	1.9912	4	5.77	1.8573
44	4	58.73	1.9912	3	47.14	1.5682	4	4.20	1.4314	3	5.53	1.4314
45	8	58.64	2.5551	10	47.59	2.4456	8	4.17	1.6232	10	5.56	1.9294
46	6	58.66	2.1847	3	47.45	2.3032	6	4.32	1.7559	3	5.68	2.1239
49	21	58.73	3.0249	17	47.55	2.3201	21	4.12	2.5391	17	5.42	2.6253
50	8	58.67	2.1430	3	47.53	2.5786	8	4.11	2.0934	3	5.61	1.3424
Theory		58.53			47.40			4.09			5.47	

Tables I and II show those variables whose effects were compared and a summary of each collaborator's data. In these tables, n is the number of analyses reported by each analyst, $\bar{X}$ is the mean of his values, and log s^2 is the log of the variance of his data. Each collaborator was asked to report all the values he obtained, after his method had been proved satisfactory by analysis of a standard compound of his choice. All data received were used in calculating the $\bar{X}$ and log s^2 values.

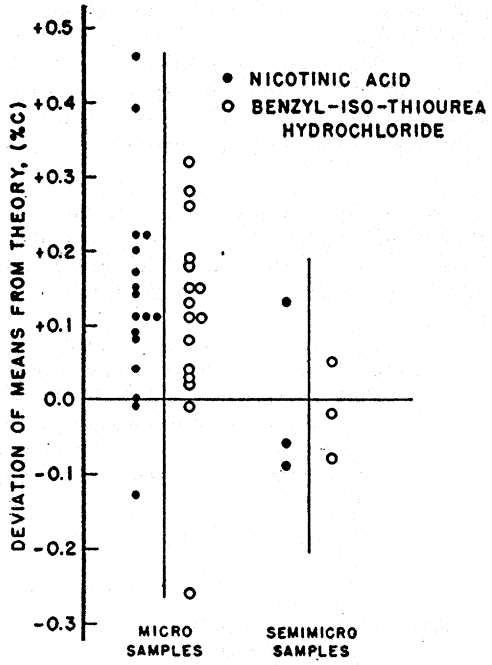

Figure 1. Effect of Sample Size on Carbon Results

In the 12 columns showing the different variables studied, × means that the analyst employed the procedure listed at the head of the column, whereas — marks the alternate procedure.

Following are the procedures listed in Table I plus the alternates with which they were compared:

Micro samples (2 to 10 mg.) *vs.* semimicro samples (10 to 20 mg.)

Absorption tubes wiped before being weighed *vs.* not wiped

Oxygen in absorption tubes replaced with air before weighing *vs.* oxygen not replaced

Use of a choking plug in exit end of combustion tube *vs.* omitting this plug

Oxygen aspirated with Mariotte bottl *vs.* pressure only to force oxygen throug system

Use of silver alone to remove sulfur oxides *vs.* silver plus lead chromate

Copper oxide plus platinum catalyst *vs.* all other combinations used

Electric sample burner *vs.* gas burner

Mechanically operated sample burn *vs.* manually operated burner

Quarts or Vycor combustion tubes *vs.* borosilicate glass and other heat-resistant tubes

Analyses conducted in air-conditioned laboratory *vs.* analyses from non-air-conditioned laboratory

Balance located adjacent to furnace irrespective of air-conditioning *vs.* balance in air-conditioned balance room

To determine whether or not there was any marked difference in accuracy, the carbon data obtained with micro- and semimicroprocedures were plotted (Figure 1) as suggested by Tukey (*2*). Inspection of this simple plot of the deviation of the means from the theoretical values readily shows that the micro values were in general higher than the semimicro. Student's t test (*1*) was applied to these data to determine if this apparent difference was really significant.

$$= \bar{x}\sqrt{\frac{n_a n_b\,(n_a + n_b - 2)}{(n_a + n_b)\,(S_a x^2 + S_b x^2)}}$$

where $\bar{x} = \bar{X}_a - \bar{X}_b$ (difference between means of two groups)

n_a = No. of values in group a, micro

n_b = No. of values in group b, semimicro

$$S_a x^2 + S_b x^2 = \Sigma(X_a - \bar{X}_a)^2 + \Sigma(X_b - \bar{X}_b)^2$$

The t value calculated from these data was 2.24 and the critical $t_{0.05}$ obtained from the table of t values was 2.03. The difference between the two means, therefore, was critical at the 95% level. This indicates that semimicromethods will produce more accurat results than microprocedures, if the data were representative and not biased by other variables.

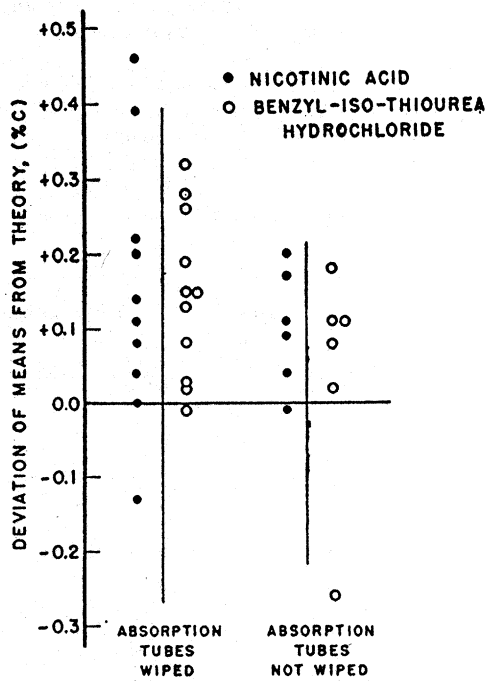

Figure 2. Effect of Wiping and Not Wiping Absorption Tubes

Because there was a significant difference between the micro and semimicro values, it seemed desirable to eliminate the effect of this variable from subsequent comparisons. Therefore, the values obtained using micro samples were treated separately, so that the effect of the other variables on the accuracy of the carbon values could be determined without bias due to the sample size.

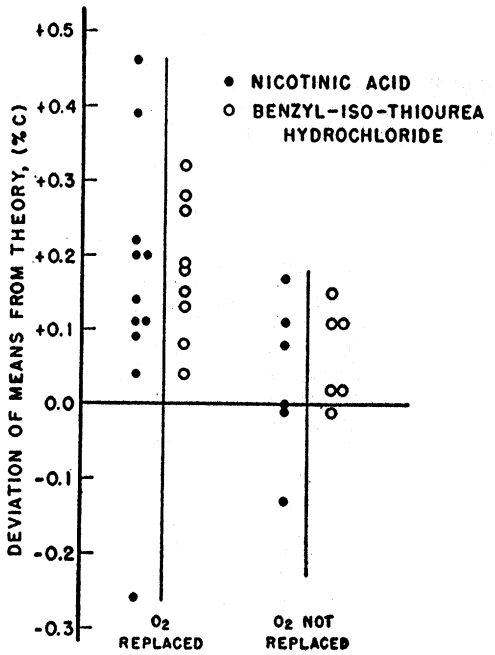

Figure 3. Effect of Replacing Oxygen in Absorption Tubes with Air

Using only micro values, the effect of wiping or not wiping the absorption tubes before weighing was analyzed by this same procedure. Figure 2 shows a plot of the data, and on inspection it appears that wiping has a tendency to cause high results. When the t test was used to determine the significance of the difference, the calculated value was 1.67. Thus the difference was significant at the 90% but not at the 95% level.

Figure 3 shows the values obtained when the oxygen in the absorption tubes was replaced before weighing and when it was not replaced. The difference that can be seen here is significant, as the t value for the difference between means was greater than $t_{0.05}$. No significant differences in the accuracy of the carbon values were found between the alternate procedures for the remaining nine variables listed in Table I.

The question arose as to whether or not the two apparently critical variables biased the micro data for the other variables, so that no critical differences appeared when in reality some actually existed. To determine this, the effect of the two variables which appeared to be important (wiping the absorption tubes and replacing the oxygen) had to be at least partially eliminated by adjusting the data. Figure 4 shows that there was considerable overlapping of the data for these two variables; seven analysts both wiped the tubes and replaced the oxygen and only three each used the other three combinations of these two variables. Adjusting the data to eliminate the effect of either of these variables also made the other variable not critical. Therefore, rather than attempting to select the more important variable, the data were adjusted to eliminate the effect of both. The median deviation of the mean from the theoretical value for the 14 carbon results obtained by those who both wiped the tubes and replaced the oxygen was +0.20%. The median for those who did neither was +0.11%, and for those who performed either one or the other operation it was +0.06. This latter value should logically fall between 0.20 and 0.11%, and the fact that it did not may mean that there was an interrelationship between the two variables or that the value was low by accident. Regardless of this, it can be concluded with reasonable certainty that the effect of both wiping the tubes and replacing the oxygen was to cause the results to be high by approximately 0.10%. To adjust for this effect 0.10% was subtracted from the values obtained by those who performed both operations. These adjusted data were then re-examined to determine if any of the previously noncritical differences would become significant. However, the only effect of any importance

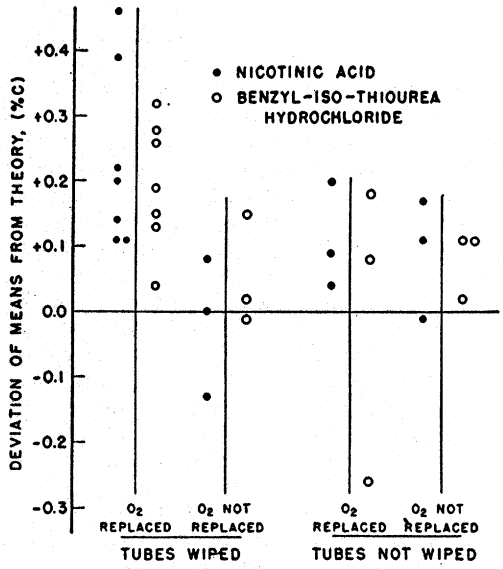

Figure 4. Effect of Wiping Tubes and Replacing Oxygen

Table III. Statistical Data for Variables Causing Critical Differences in Carbon and Hydrogen Results

	Comparison	n	$\bar{X}$	Sx^2	$t_{calcd.}$	$t_{0.05}$
Carbon	Micro samples (2–10 mg.)	34	+0.12	0.6149	2.24	2.03
	Semimicro samples (10–20 mg.)	6	−0.01	0.0384		
	Absorption tubes wiped[a]	22	+0.15	0.4073	1.67	2.04
	Absorption tubes not wiped[a]	12	+0.07	0.1630		
	Oxygen replaced with air[a]	20	+0.17	0.4233	2.53	2.04
	Oxygen not replaced with air[a]	12	+0.05	0.0800		
	Tube wiped and O_2 replaced	14	+0.21	0.1695	2.38	2.10
	Tubes not wiped and O_2 not replaced	6	+0.09	0.2325		
	Micro samples[b]	32	+0.09	0.4846	1.94	2.03
	Semimicro samples	6	−0.01	0.0371		
			log s^2			
	Oxygen replaced with air	20	1.87	7.148	2.16	2.03
	Oxygen not replaced with air	18	2.33	8.227		
Hydrogen	Oxygen replaced with air	20	1.76	7.648	2.31	2.03
	Oxygen not replaced with air	18	2.15	2.050		
	Oxygen aspirated (Mariotte bottle)	20	1.76	7.708	2.58	2.03
	Pressure alone	20	2.15	1.935		

[a] Micro data only used in calculations.
[b] Micro data corrected by subtracting 0.10 from values obtained when tubes were wiped and oxygen replaced with air.

was on the micro-semimicro comparison and this was to reduce the difference, as only micro values were lowered by adjustment of the data. The t value using the adjusted data was 1.94, which is still critical at the 90% but not at the 95% level.*

The t values of importance for the whole study and the data necessary to calculate these values are shown in Table III.

In addition to the effect on accuracy, it was desired to know the effect of the variables on the precision of the results. A method for doing this, suggested by Tukey (*2*), consisted of determining the variance of each analyst's carbon values, taking the logarithm of these, and applying Student's t test as if they were means. When this test was applied to the carbon values, only one significant difference (95% level) in precision appeared. As 11 separate tests were made, one would be expected to appear critical about half $[1-(0.95)^{11}]$ of the time. This critical difference was for oxygen in the absorption tubes replaced with air before weighing as compared with not replacing the oxygen. The calculated t value was 2.16 as against a critical value of 2.03 at the 95% level. This particular variable apparently had the greatest effect on the accuracy and on the precision of any of the variables evaluated. While replacing the oxygen caused significantly better precision, it also caused significantly poorer accuracy, showing that the two criteria by which data are judged are not the same or related, even when working with a large number of values.

Information concerning the effect of the variables on the accuracy and precision of the hydrogen data was also desired. The same tests were applied to the hydrogen data as to carbon. None of the variables appeared to have a significant effect on the accuracy of the hydrogen determination, although two variables affected the precision. One, as in the carbon study, was whether or not the oxygen in the absorption tubes was replaced with air before weighing, and the other was whether pressure plus aspiration with a Mariotte bottle or pressure alone was used to drive oxygen through the combustion train. Table III shows the calculated and critical t values for these variables.

CONCLUSIONS

The purpose of the study was to determine which combination of techniques should produce the best carbon and hydrogen results. To do this, it was necessary to arrange the desired objectives of a good method in the order of their importance. The following order was used:

Accuracy of carbon results
Accuracy of hydrogen results
Precision of carbon values
Precision of hydrogen values

In addition to these objectives, such items as the complexity of apparatus and technique, the number of determinations possible per apparatus per day, amount of the analyst's time spent per analysis, and the possibility of simultaneous operation of two apparatus were also considered. With these objectives and consideration in mind, a study of the result led the authors to conclude that the carbon and hydrogen procedure should include the following: electric furnaces with mechanical operation, quartz or Vycor combustion tubes with a filling of copper oxide plus platinum catalysts and silver wire or ribbon, no choking plug, pressure only for oxygen flow; no treatment of the absorption tubes other than to allow them to equilibrate before weighing; and use of samples weighing 10 to 15 mg. if possible.

Directions for a method including these features were written and submitted to a number of collaborators, so that the conclusions drawn from this study could be tested. The results will be published in a referee report to the Association of Official Agricultural Chemists.

ACKNOWLEDGMENT

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(2) Tukey, J. W., private communication.